Gene Expression Profiling: qPCR Toolkit for Quality Control

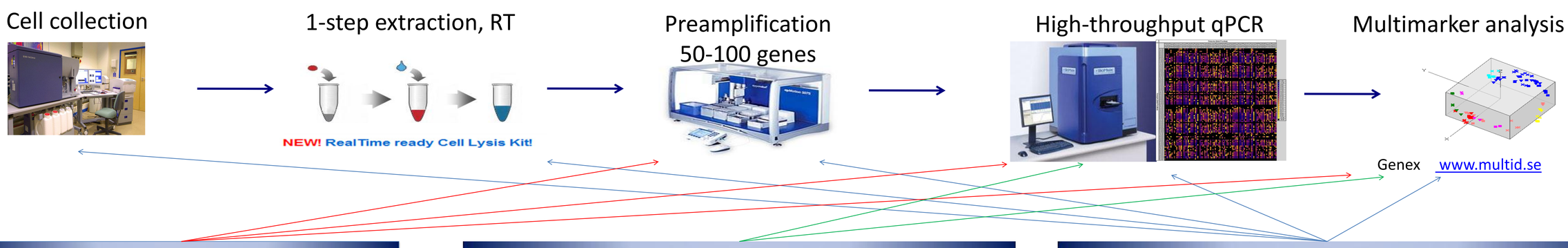
Švec D. [1], **Jacobsson J.** [1] , **Sjöback R.** [1] , **Kubista M.** [1,2]

TATAA Biocenter AB, Gothenburg, Sweden[1]; Dept. Of Gene Expression, IBT, Academy of Sciences, Prague, Czech Republic[2]

David.Svec@tataa.com

Gene expression profiling is exceedingly important tool in functional genomics research and in drug discovery. The general trend in the field is towards higher throughput of less complex samples. Nowadays studies are appearing where expression of tens of genes is measured in hundreds of individual single cells, to be able to characterize cell subtypes and its roles. To identify subpopulations of astrocytes, to define them in molecular terms, and to determine the biological progress of astrocyte maturation on single cell level we developed and optimized protocol for high-throughput gene expression profiling using qPCR together with a system of quality control: **ValidPrime™** - control for genomic DNA background, **Interplate calibrator -** tool, which is used to compensate for inter-run variation in qPCR and **Universal Spike (RNA/DNA)**, which can be used to detect presence of inhibition in samples of any living species or monitor losses and yield of isolation, or check the itegrity of mRNA during sample processing, storage and transporation.

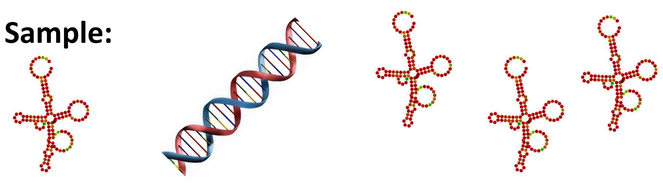
Workflow:



ValidPrime™

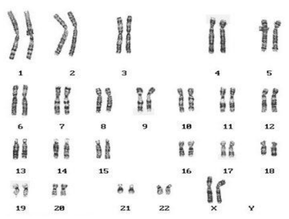
ValidPrime™ is an assay to test for the presence of gDNA in test samples and when combined with a gDNA reference sample, replaces all RT(-) controls. ValidPrime™ is highly optimized and specific to a non-transcribed locus of gDNA that is present in exactly one copy per haploid normal genome. Therefore, ValidPrime™ measures the number of genomic copies present in a sample and can be used for normalization of samples to cell copy number, as endogenous control for CNV applications, and as control for gDNA background in RTqPCR.

Sample:



Target gene Cq = X mRNAs + Y gDNA copies (incl. repeats, pseudogenes)
ValidPrime Cq = number of gDNA copies (haploid genomes) in sample

Reference standard (gDNA):



Target gene Cq = hits per gDNA including repeats, pseudogenes..
ValidPrime Cq = number of gDNA copies (haploid genomes)

Subtracting DNA contamination from mRNA signal:

$$Cq^{TG}_{RT^-} = Cq^{TG}_{gDNA} + \left(Cq^{ValidPrime}_{Sample} - Cq^{ValidPrime}_{gDNA}\right)$$

$$Cq^{TG}_{mRNA} = -\log_2\left(2^{-Cq^{GOI}_{RT^+}} - 2^{-Cq^{GOI}_{RT^-}}\right)$$

More applications:

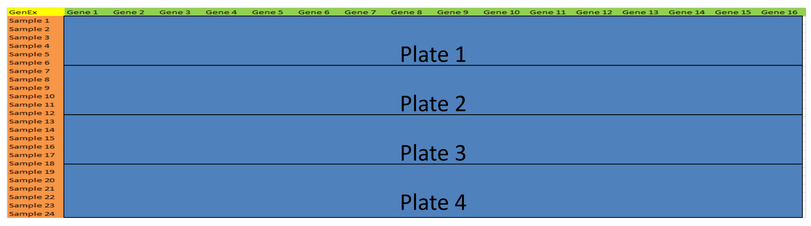
- Normalization per cell (number of genomes)
- Compare expression between different species
- Normalizer in copy number variation (CNV) studie

Interplate calibrator

Interplate calibrators are used to compensate for variations between runs due to instrument settings (base-line correction and threshold settings). These variations are independent of assay, but depend on instrument channel used. The Cq of an interplate calibrator must be determined with very high accuracy, else interplate calibration may add more variance to the data than the systematic variation it removes. It is highly discouraged to perform independent inter-plate calibrations per assay!

When expression of genes and samples is compared multiple runs can be merged for common analysis without correction if either:

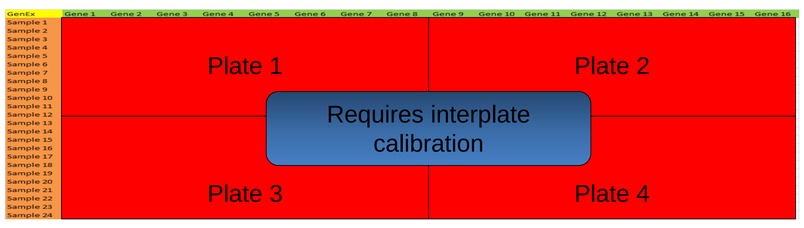
- All genes analyzed for each sample are assayed in the same plate ("All genes")



OR

- All samples analyzed for each gene are assayed in the same plate ("All samples")

if none of that is possible – MIXED analysis:

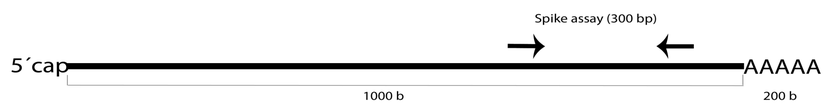


Interplate calibrator is:

- very robust assay, SD of triplicate < 0.1 Cq
- uncomplicated and stabilised template at fairly high concentration (15 <Cq < 20)
- provided in 50 aliquots ready to use (-20°C storage)
- ☐ IPC should be run in replicates (minimum triplicates)
- ☐ Note that one IPC per channel is sufficient!

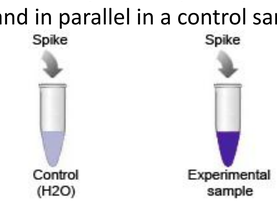
Universal RNA Spike™

The Universal RNA Spike is simple and very efficient tool for quality control of RNA samples prior to RT-qPCR. The Universal RNA Spike has a sequence, which is not present in any known living organism, the length is 1000bp and the spike consists of poly-A tail (approximately 200bp) and 5´ Cap. The Universal RNA Spike is therefore a perfect mimic of eukaryotic mRNA, but without homology to known nucleic acid sequences, which makes TATAA spike convenient to use for samples from any species. One assay has been designed and tested to detect inhibition, another system of assays is optimised to check integrity of mRNA strand either based on different lenghts of amplicons or the 3´/5´ ratio. This is useful to test stability of RNA in buffers, lysis reagents, validating long term stored nucleic acids or monitor integrity of FFPE material.

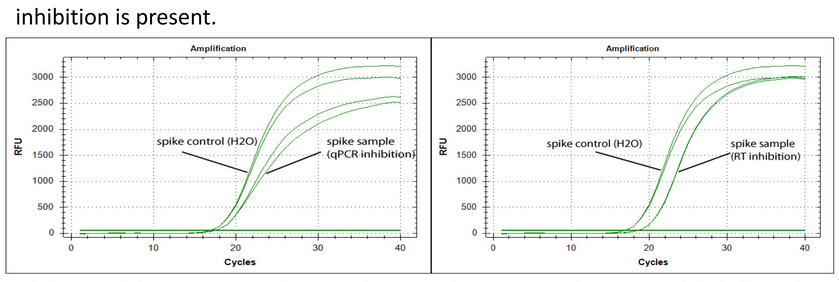


Detection of inhibitors:

Test for inhibition is based on a simple principle: equal amount of spike is added in the experimental sample and in parallel in a control sample containing pure water.



Both sample and control are reverse transcribed and amplified using the Spike Inhibition assay. If the Cq value of experimental sample is higher than control, the inhibition is present.



If the amplification curve shows a decreased steepness than control (left fig.), the inhibition is very likely to be present in qPCR and all upstream enzymatic reactions. If the Cq value differs and the shape of amplification curves is identical for sample and control (right fig.), the inhibitor might have been diluted sufficiently between reverse transcription (RT) and qPCR, but inhibition still was present in RT.

Innovative Techniques and Models to Study Glia-Neuron Interactions

This work has received funding from the European Community's 7th Framework Programme through the Marie Curie Initial Training Network Edu-GLIA (PITN-GA-2009-237956)